THE

# VETERINARY JOURNAL

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NOVEMBER, 1940

## Editorial

In the control of contagious diseases in domestic animals early diagnosis is essential. The recognition of clinical symptoms and lesions, especially in the less acute diseases—and this is sometimes a matter of difficulty except to those with adequate experience—means that the infection has become established and that in all probability the affected animals are at that time and may have been for some time past excreting the causal micro-organisms or viruses resulting in spread of the disease. Even in parasitic infestation similar remarks are true. It is essential, therefore, that wherever possible other methods of diagnosis should be available in order to detect infected stock before clinical symptoms appear. An outstanding example of a diagnostic method is the use of tuberculin. Tuberculin testing has been practised for many years and though setbacks are experienced from time to time, it must be admitted that, generally speaking, by its practice much progress has been made in the control of tuberculosis. The subcutaneous test has been replaced by the intradermal method and new types of tuberculin have been evolved. It has been amply demonstrated that by the use of tuberculin and the correct interpretation of the results, tuberculosis can be cleared from herds of cattle following which steps can be taken to maintain their freedom from the disease. It is essential that the tuberculin used in such tests be of a degree of potency

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and the dose injected be such that bovine tuberculosis will be detected in the maximum number of infected animals into which it is injected. It is necessary, also, that the type of reaction produced is of such a nature that its interpretation is not merely a matter of opinion but is agreed by all experienced persons. The time is not far distant, we hope, when difficulties experienced in the past will be obviated and the interpretation of the reactions following the use of tuberculin will be simplified.

In some parts of the country Johne's disease causes heavy losses in cattle. It is now agreed that animals may be infected and may be spreading infection for considerable periods before clinical symptoms are apparent. The use of Johnin is not being practised to the same extent as is tuberculin: it is hoped that in time, with further research work, Johnin of an accepted type and standard will be available when its use will be a routine matter in the control of cattle disease. In the meantime, other methods of diagnosis have been studied and following the report of work in America, one of those methods has been further investigated in this country. We publish elsewhere in this number two articles on the use of laboratory animals—guinea-pigs, rabbits, mice and fowls—in diagnosing infection with B. johne. From the early reports of the method it appeared that a comparatively simple method of diagnosing the disease had been evolved, but there would now appear to be some doubt of the specificity of the test. The lesions developing in laboratory animals following their injection with liquid paraffin emulsions of the causal organism can apparently be set up by other acid-fast organisms and even when they have been killed. Any test for the detection of an infection must be specific. It is important, therefore, that in this method of testing, further research be carried out before it can be accepted as of value in the diagnosis of Johne's disease.

Research workers are well aware of the necessity for the repetition of experiments before concluding that their results can be generally obtained. There are so many pitfalls in working out methods of diagnosis that it behoves all who make claims to ensure that they are specific and will yield similar results in the hands of different observers.

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## General Articles

# THE PATHOLOGY AND PATHOGENESIS OF TUBERCULOSIS IN ANIMALS COMPARED WITH MAN

(*Continued.*)

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### Bovine Tuberculosis*

MANY views in this country of the pathology and pathogenesis of bovine tuberculosis appear to be based on the authority of veterinary papers written at a time when the disease in man was regarded in a different light than prevails at present. The distinction between the phases of disease in the child and adult has been slowly appreciated and applied to the bovine condition, and old pathological concepts are still tenaciously held by the veterinarian. As a brief introduction some attention is given to aspects of bovine tuberculosis which require more and fresh consideration.

A thorough study has not been made of the differences and significance of tissue reaction in the same and different animals to different types of tubercle bacilli and the various phases of the disease development. It is thus insufficiently stressed that a tuberculous lesion is not synonymous with caseation and that a macroscopic examination alone is often not enough to establish a diagnosis. Certain changes which occur in lymph nodes in man, described originally by Ziegler as "large cell hyperplasia," and known now as chronic proliferative non-caseating tuberculosis, may not be easily recognised macroscopically or may even escape detection. So far studies of this entity have not occupied much space in veterinary literature; yet it occurs in certain phases of bovine tuberculosis, frequently in the dog, and has a classic exaggerated example in equine tuberculosis. In medical literature, on the other hand, it

* It was intended that this part should be treated as fully as the first section and with ample illustrations. A grant was obtained in 1939 for this purpose: the war and change of occupation interfered with this project and it is unlikely that it will ever be done. Rather than leave the publication uncompleted it was thought that the form it now takes might be of some value, partly to indicate wherein further knowledge must be sought of a disease which we are so busy eradicating by methods of extinction of the host.

has received much attention, particularly in regard to the relation of associated atypical processes (e.g., Boeck's sarcoid, benign lymphogranulomatosis, multiple benign sarcoidosis—which may all be names for the same condition) to active tuberculous infection. A general opinion is that these atypical forms are identical in essence and are simply different manifestations of the same morbid process caused by tubercle bacilli and that their pathogenesis is a particular phase of tuberculosis. It must be remembered that there can be no rigid dividing line between non-caseating tuberculosis and caseous processes and while type forms are clear cut, transformations are frequently found.

Remarkably little is known about the animal aspect, but the atypical skin lesions of bovine reactors (e.g., see Hole, 1938) might come into this category. The illustrations of some lesions certainly show resemblance to some Boeck's sarcoid.

There is abundant proof that the lesion of primary infection in man and animals may be microscopic in dimension and may be frequently missed;† naked eye examinations may thus fail to detect a lesion of size and, from what has been said in the preceding paragraph, a lesion of kind. The size of the bovine organs, in particular the lungs and intestines, renders thorough examination difficult. Nevertheless this difficulty must be overcome when autopsies are made for scientific investigations. In addition to the possible minute size of primary infection, it has been mentioned how a certain kind of lesion might escape macroscopic recognition. One wonders, therefore, how many papers in the past dealing with animal tuberculosis have been guilty of such omissions. Ample proof of the importance of these points can be produced. For example, in the protocols of experiments by Nieberle instances are given of calves in which, although macroscopic caseous lesions were found only in the lungs or pulmonary lymph nodes, a non-caseating proliferative primary lesion was found on histological examination in the tonsils or tonsillar lymph nodes. The necessity for histological examination as an adjunct to all autopsies made for investigating the mode of infection of tuberculosis and its spread within the body must be clearly recognised. From the above, possible sources of error are apparent when statements are made of the ability of bacilli to pass through tonsils, lungs, or intestinal mucosa, etc., of cows and to leave no trace of their passing. This may simply indicate that the author did not observe *macroscopic changes* and that conclusions regarding the genesis of the disease based on macroscopic grounds alone have little value. In spite of this there is a wide belief that tubercle bacilli can pass through the intestinal mucosa, cause no lesion, and by a devious path reach and produce lesions in their final resting place—the bronchial or mediastinal lymph nodes. Many believe that this is the mode of

† Terplan (1940) carried out meticulous anatomical studies on tuberculosis in human beings and stated that, if time and again foci are not found, this by no means proves they were not present; most probably they were in a state hardly recognisable by casual anatomical examination.

infection in calves which show isolated macroscopic lesions in these glands. Gofton (1937), dealing with infection in the calf, for example, states: "The fact that in such a large proportion of cases the tuberculous lesions are confined to the lung glands is *definite evidence* (italics mine) that the tubercle bacilli can and do become established in situations within the body without leaving the slightest trace of the point of entry or the route which has been traversed to reach this situation." Further that: "Although *I cannot prove it* I strongly suspect that quite a number of cases, in which there are primary lesions confined to the bronchial or mediastinal glands, have their origin in alimentary infection." No proof has ever been offered to support statements like these and the mere fact that the lesions are isolated is no evidence of the mode of infection. It has also never been explained by what mysterious anatomical path the bacilli can pass from intestinal mucosa to the thoracic cavity. While there are afferent paths of lymphatics from the peritoneal cavity through the diaphragm to the other cœlomic cavity, it surely cannot be imagined that bacilli pass through all coats of intestine, to reach the peritoneal cavity, traverse this and then pass through the diaphragm. The alternative explanation would be that the bacilli pass through the intestinal mucosa —mesenteric node—thoracic duct—lungs—bronchial nodes, surely a long, peculiar route to travel in order to set up pathological changes only in the latter. Yet these are the theoretical highways if one attempts to find possible anatomical support for this belief. My own opinion is that in this matter we are asked to believe something which approaches the incredible without any scientific proof. The time is appropriate when these beliefs should be examined critically and either valid support brought forward or the ideas heartily condemned. Recent work by Edwards (1937) was also believed to supply ample supporting evidence of this mode of infection in calves which were fed by mouth with infected milk and in which, at autopsy, macroscopic lesions were found only in the bronchial and mediastinal glands. The author admitted that the experiments were not carried out for the purpose of studying the pathogenesis of the disease and therefore microscopic studies were not considered. From what has been said previously decisions like those made in this case on macroscopic evidence alone have very problematical value. In a parallel feeding experiment by Nieberle while *macroscopic* lesion were detected *only* in the bronchial nodes, histological examination of the tonsils and retropharyngeal nodes revealed non-caseating proliferative tuberculous lesions. Nieberle's conclusion was, with good reason, that the mode of infection was primarily through the naso-pharynx from the mouth and that, later, or simultaneously, bacilli had been aspirated from the mouth to the lungs and then from the latter to the bronchial glands—quite a different and fundamentally important deduction from that of Edwards and one which emphasises the great dangers of aspiration from the mouth as a source of pulmonary infection. It was claimed by Edwards that the method he adopted of feeding

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infected milk to the calves obviated the danger of aspiration, but surely once fluid reaches the mouth there is nothing to prevent the possibility of aspiration. There is a noteworthy, but tragic, example of this danger of aspiration infection in the Lubeck disaster where a nurse admitted, in her evidence, to holding the nostrils of eight children to make them swallow the "BCG vaccine": six out of the eight thus treated developed *multiple primary foci* in their lungs.

We still require more data regarding the lymphatic system* in the cow and until more is known, many views on pathogenesis will be disputed. Retrograde infection by lymph paths has been a favourite theme of debate for years although the evidence brought forward has no real solid foundation. There appears to have been little recognition of the fact that lymph vessels, even the smallest, possess valves which permit the flow of lymph only in one direction. Retrograde circulation of lymph and transport of bacilli can occur therefore, in view of the rich anastomoses of lymphatics, only if stasis is present over a very wide area. This must be a rare event in tuberculosis and it appears that much of the discussion on retrograde paths of spread seems to have occurred simply because no other explanation was obvious. Some of the older experiments, designed to prove the ease of occurrence of retrograde lymphatic infection in the cow, did not take into account the existence of the rich anastomoses of the afferent bed (from distant sources) of lymph nodes. For example, the spread of infection from near the occiput and upper part of the neck to the prescapular node, in one experiment made many years ago, was concluded to be retrograde spread of the disease, but this is one of the normal afferent passages of lymph to the gland according to Baum! A better knowledge of the anatomy of lymphatics might also settle conclusively the disputed origin of infection of young calves showing isolated liver or portal gland lesions.

There is thus great scope for more thorough pathological investigations along the lines indicated above if the disease is to be fully understood. A more critical attitude is certainly required in considering the disease in the cow and we must free ourselves of dogma fostered by bygone authority. Routine autopsies of bovine animals have never been made on the same large scale, nor in the same systematic manner as of human beings in

* Harris (1937) stated that most illustrations of lymphatic vessels and lymph nodes in standard textbooks of human anatomy are variants of the beautiful plates in Mascagni's famous atlas, published in 1787. Successive anatomists and artists, often with more imagination than accuracy, continue to depict in green various lymphatic vessels and glands in relation to veins coloured blue. One wonders to what extent our knowledge of the lymphatic system in animals may be parallel. The highly important work of Batson (1940) shows that we must revise our conceptions of the venous system. According to him there is, in man, a large group of veins along the vertebral column which act as a separate system to that of the pulmonary, caval, and portal, which can act as a by-pass thereby allowing direct spread of tumours and abscesses anywhere along the thoracico-abdominal wall. This system provides a ready explanation of paradoxical metastases in tumours as it removes the stumbling-block of the necessity of lung involvement.

which great help is obtained by previous clinical and X-ray examinations and by subsequent histological studies. More and extensive data of bovine tuberculosis—clinical, anatomical and histological—are required regarding (a) the sites, incidence and fate of primary infection, (b) the proportion of healed cases, (c) the occurrence of "silent entries" of the bacilli—if they do occur, (d) the modes of dissemination of infection in the body, and (e) the reaction of the calf and adult cow to different types of bacilli and to super-imposed re-infection. The various anatomical changes in the disease of the calf and adult require clear definition of their type, nature and pathogenesis: this is highly necessary as is evident from the confusion that exists regarding what constitutes acute and chronic miliary tuberculosis, which in the case of the lungs appear to be mistaken frequently for localised disseminations. Comparative studies are required regarding the distribution of lesions, for example, in the lungs and udder, in order that we may understand why there appear to be predilection sites for the development of certain phases of the disease. In all these respects conclusions based on meat inspection examinations of carcases in slaughterhouses, however excellent, cannot obviously take the place of those derived by the autopsy work of the pathologist.

A foundation and example lies before the veterinarian in the enormous amount of data which has become available by the investigations of the medical worker concerning the disease in man.

### Primary Infection in Calves

Much work has been done in recent years, mainly by Nieberle and his pupils, on the pathogenesis of bovine tuberculosis. These studies have been presented in various papers listed in the bibliography and more recently in a monograph containing valuable coloured illustrations. Many of the facts concerning tuberculosis in man, which have already been discussed, have a clear analogy in the bovine disease.

Primary infection in the calf is restricted mainly to the respiratory and alimentary systems: other sites have been recorded occasionally, such as the skin, vulva, vagina, teat and penis, but they must be regarded as rareties. A number of these cases must come into the category of accidental introduction of primary infection, e.g., through the teat by way of an infected bougie. Under primary infection we must also include congenital disease.

The primary lesion in the lungs exhibits all the characters already described in the case of the child and dog: often, but not always single, it may be found in any part of the lung with a predilection for subpleural sites in dorso-caudal parts. The size of the initial focus varies, which may depend, as calves are often killed, on the stage of the disease at which it is seen. Multiplicity of primary lesions must be distinguished from a single focus with satellite tubercles in the immediate vicinity. The lesion conforms in histological structure to the classic tubercle in which central caseation may be marked,

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epithelioid cell reaction distinct, giant cell formations abundant and calcification may occur at an early stage. The lesion may become encapsulated by fibrous tissue and be sharply demarcated from adjacent normal lung substance. With regularity the regional pulmonary lymph nodes show caseous and calcified lesions and their location in early development can be related to the lymph drainage area of that part of the lung in which the primary lesion is situated. The complex may thus be complete, although it is said that frequently the regional lymph nodes only may be involved. Whether this indicates (a) that the bacilli have passed through the lungs without producing any lesion, (b) that a minute lesion had been produced but had resolved completely, or (c) that macroscopic examination failed to detect it, is unknown. I incline to emphasising the last two explanations as I believe that the possibility of small tuberculous lesions undergoing complete resolution has never been sufficiently realised. In any case, complete or incomplete, primary lung complexes may be found in calves as young as 3-4 weeks, but it must be stated here that there are no criteria available for the accurate determination of the age of a lesion. Minute discrete lesions may be found in other parts of the lungs of such cases which may indicate (a) multiple primary infections, (b) early discrete hæmatogenous spread within the pulmonary circulation, or (c) early spread by bronchogenic aspiration.

Primary infection via the alimentary canal includes cases in which the site of entry is the tonsil and small intestine, although the exact incidence is not known. The possibility of frequent primary tonsillar infections has not been appreciated until lately as histological examination may be necessary to establish a diagnosis. Intestinal primary infections, said to be more frequent in the region of the ileo-cæcal junction, appear to result commonly in incomplete complexes. Again, however, recent work has shown that histological examination of Peyer's patches in parts of the intestine related to the involved lymph nodes may reveal minute tuberculous foci. The enormously enlarged caseous and calcified nodes which may be found in association with primary alimentary infection in calves is a well-known observation.

*Congenital primary infection.*—This arises in all cases by transmission of the bacilli from an infected uterus to the placenta and thence to the fœtus. It needs only a brief consideration of the anatomical pathways between the fœtus and the mother to realise how infection can occur and as a result in which organs of the very young calf isolated primary lesions might be located. The calf might be infected at birth by aspiration of infected amniotic fluid into its lungs or by swallowing it. The occurrence of an isolated pulmonary or alimentary focus in a calf up to the age of 3-4 weeks cannot therefore exclude such a possible post-natal source. I doubt whether any authentic cases have been recorded. The fœtal calf might also be infected *in utero* by transport of the bacilli along the umbilical vein into the fœtal circulation, in which case foci would be expected to develop in the liver or portal lymph nodes — a

common enough finding in newborn and very young calves. It was formerly held that, when isolated lesions were found in these tissues of calves over 14 days old, it was impossible to decide whether such infection had been congenital (*via* the umbilical vein) or had been a case of infection by ingestion after birth. The site of entry of the bacilli in the latter event was considered to be the duodenum since it was assumed that the portal gland also drained the first part of the small intestine. Nieberle showed that when bacilli were fed in milk to experimental calves, in no single instance were lesions found at post-mortem examination in the portal lymph node. It is now contended by Nieberle that all calves, no matter what age, which show isolated lesions in the liver or the portal gland are examples of true congenital infection. In some parts of Germany the mothers of such cases were followed up and examined: a survey has revealed a high percentage of chronic uterine tuberculosis. There remains the possibility of bacilli entering the umbilical vein and being short circuited by the *ductus venosus* directly into the caudal *vena cava.* If this occurred at a late stage of gestation it is possible that a primary lung focus could be produced. The fœtal lungs, however, are collapsed which might preclude the development of disease, and as a result the bacilli might be short circuited into the fœtal systemic circulation and produce single or multiple lesions in the viscera. (A good example of the necessity of a free circulation in the lungs for the production of tuberculous lesions [hæmatogenous in origin] is shown by the case illustrated in Fig. 42 where miliary tuberculosis occurred in the left lung but not in the right which was collapsed by pleurisy.) This, in fact, may be the explanation of the apparent paradoxical distribution of lesions in some congenital calf cases in which there are disseminated lesions involving liver, spleen, kidneys and symmetrical peripheral lymph nodes.

The figures given by Nieberle of primary infections in calves in Germany are: congenital routes, 48 per cent., respiratory routes, 39 per cent., and alimentary routes, 13 per cent. Accurate comparable data for this country are not available, but in any case the criteria of anatomical diagnosis used by Nieberle have never been adopted in this country.

**Generalisation of the Disease after Primary Infection**

The primary complex (complete or incomplete) in the calf, as in the child, may heal by fibrous encapsulation and calcification and thus become an obsolete infection, although in what percentage of bovine cases this occurs, or whether apparent obsolete lesions undergo recrudescence in later life is unknown. Complete healing of the primary infection in man may be followed by disappearance of the former positive tuberculin reaction. Similar events might occur in the bovine animal but clinical and anatomical data have not been produced. Without healing of this primary lesion the bacilli begin a journey which may carry them far and wide through the body. The path is primarily

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towards the lungs, the latter acting as the great distributing centre for all subsequent disseminations.

From the initial focus infection may spread by (1) direct contiguity, (2) intracanalicular, (3) lymphatic, and (4) hæmatogenous paths.

1. An example of the first is the way in which a caseous focus extends in a centrifugal direction, thus becoming ever larger and larger, or produces a collection of satellite tubercles in the immediate vicinity. In the body cavities a parallel process of contact infection must undoubtedly be responsible for the production of tuberculous pleurisy and pericarditis from lung or thoracic lymph gland sources, of peritonitis from comparable regional sources, while the genesis of meningitis by the breakdown of a brain focus into the meningeal spaces is very similar.
2. In an organ such as the lung there is a constant danger of the infection spreading along the natural channels. Aspiration, as well as local extension, can thus spread the disease from alveoli to alveoli and from these to bronchioles and bronchi so that the process, at first lobular, may assume lobar proportions. In addition bacilli may be coughed up in sputum to be implanted on trachea, larynx and mouth or even be swallowed to produce intestinal tuberculosis—common events both in man and the cow. As these organs are simply appendicular to the bronchial system, disease spread in this way is not a true example of extra-pulmonary generalisation. This intracanalicular spread of infection is, however, a greater characteristic of the chronic organ tuberculosis of the adult than of early generalisation.
3. Bacilli, or phagocytic cells containing the organism, are transported at an early stage along the lymph vessels from the primary organ focus to the regional node. The same process may be repeated to other nodes in the direct afferent path of lymph circulation, hence a chain of glands may be involved. The greatly enlarged caseous, partly calcified, lymph gland lesions, so often seen in the mesenteric and mediastinal groups in calves, are typical examples of the disease evolving in this manner.
4. Bacilli rapidly and easily penetrate into the blood from tissues, although this does not necessarily presuppose a septicæmia. In small numbers they must soon disappear from the blood to be taken up again by tissues, where they may either produce a focus, or fail to propagate. Such a transient bacteriæmia seems, at least in man, to be an invariable sequel to primary infection, while experimentally it has been shown that this passage into the blood for a temporary stay may be a rapid one.

As all possible transport of bacilli via the lymph vessels must eventually reach the systemic circulation through the lungs, we may speak of a lympho-

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hæmatogenous spread of infection which appears to play a big role in bovine tuberculosis.

The forms of dissemination and the factors which limit this may now be considered. From a primary lung focus, or from secondary involvement of the lungs from alimentary infection, there are two possible sequels, namely, extension of the disease within the lungs or through the systemic circulation. We shall not consider cases in which the disease process becomes inhibited or even latent. A bacillæmic invasion of the blood is possible at all stages, which need not be fatal and which may have an origin in the lung itself or in extra-pulmonary sites. The bacilli may be destroyed in the blood or in the tissues receiving them, or may settle in distant organs to produce isolated, perhaps abortive, lesions, or may produce widespread, even universal, fatal disseminations. The factors limiting these events seem to depend on the numbers of bacilli liberated into the circulation and on the condition of the tissues which allows, or prevents, their free multiplication. One of the most important complications at this stage must therefore be acute miliary tuberculosis, about which there appears to be much confusion in animal tuberculosis. Some attention might therefore be given to a discussion of the constitution of this pathological entity.

The term—miliary tuberculosis—was originally applied in pathology simply to tuberculous lesions of a certain size, namely, that of a millet seed, and originally may not have implied massive infection of the blood stream. It concerns purely hæmatogeneous generalisation; it is therefore advisable to make it clear that the disease subsequent to primary infection is either limited to that site, or that it is generalised, in which case more than one organ is involved; only those cases can be labelled as *generalised* in which *progressive* lesions are observed in several organs. Miliary tuberculosis does not usually occur along with chronic isolated pulmonary disease of the adult man, although it is always a possible event providing the invasion of the blood stream by bacilli is sufficiently heavy. Some authors state that it is impossible to draw a sharp line between the profuse studding of organs with tubercles, spoken of as miliary tuberculosis, and more sparse scattered disseminations. For our purpose acute miliary tuberculosis must be regarded as a process characterised by the simultaneous formation, in several organs of the body, of foci which are more or less of equal size and which have similar pathological features. This is the result of a tuberculous septicæmia. The traditional view was that the process was evoked by the eruption of a caseous or liquefying focus into a vessel, or by an actual caseous endangittis, both of which might liberate large numbers of bacilli into the circulation—a view which has been largely abandoned as such an origin is not constantly found. As one author states, acute miliary tuberculosis is little different from the result of a streptococcal septicæmia in which the pathologist does not strain to find a focus rupturing into a vessel but is content to find a pin-prick, for example,

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in the finger. Eruption into vessels, intimal lesions and passage of the tubercle bacilli into the blood stream, like any other virulent organism, all may play a part in widely distributing the infection. Transmission of the bacilli may obviously occur suddenly, or successively in showers, but this does not always indicate that the different sizes of tubercles represent lesions produced by successive blood infections, or that the smaller nodules are early forms. This process need not be acute, and chronic miliary disseminations in man may persist for a long time although they may be confined to the lungs. The acute miliary process occurs mainly after primary infection in which there is progressive caseation, may be a self-propagating process and once established is usually fatal. The lesions in the lungs are usually evenly distributed throughout all lobes (see Fig. 43) although certain areas may show a greater number (for example, in the apices) and the nodules may be confluent. Disseminations in other organs may be universal although histological examination may be necessary to determine this. In the chronic miliary type the restriction of the foci to certain areas of the lungs, e.g., apex and sub-pleural regions, may be conspicuous. Acute miliary lesions may be inter- or intra-alveolar in position, and as a rule show all the features of the classic tubercle with central caseation and with exudation and hyperæmia in adjacent lung alveoli. Chronic miliary foci are more sharply defined and with a tendency to a more proliferative fibrotic reaction. Both may be accompanied by emphysema. The discrete lesions of a miliary process must be distinguished from local disseminations produced by bronchogenic aspirations, which I believe are often confused with each other in the anatomical diagnosis of bovine tuberculosis.

Much more study is required regarding the disease in the calf, but a typical acute miliary tuberculosis, in the sense used above, does undoubtedly occur and is characterised by the even diffuse studding of the lungs by minute opaque nodules. All lobes are affected but, as in the child, the distribution may be more marked in some areas (dorso-caudal parts, for example) than others. Tubercles are also found in the liver and kidneys which, however, may be found only after histological examination. The histological features are similar to those described above. According to Nieberle a much more common terminal phase of dissemination in the calf is the condition to which he gives the name of multiple lobular caseous pnuemonia. The lungs are profusely infiltrated by irregular caseous foci, larger than those seen in the miliary type and with a much greater tendency to confluence. Evidence that this is a form of hæmatogenous spread is found by the simultaneous occurrence of microscopic tubercles in the liver and kidney and in the lungs apart from the grosser macroscopic lesions. He regards this as a special atypical form of acute miliary tuberculosis: the reasons for its occurrence have not been explained. It bears some resemblance to "miliary pneumonia" of man.

Bacillæmic invasion of the systemic circulation may be mild in extent, the organisms may not survive either in the blood or in the tissues receiving

them, or discrete lesions may be set up in organs widely removed from each other. These chronic disseminations found at this stage may result in large nodular lesions with progressive caseation and no organ or tissue in the body apart from skeletal muscle (and it is not a total rarity in this tissue) appears to be exempt from this possible complication. Chance may play some part in deciding which tissues are affected, but anatomical and physiological factors must also play a role. The occurrence of isolated nodular foci in the liver, spleen, kidneys, bones, brain and in symmetrical or asymmetrical peripheral lymph nodes are well-known observations. Consequent to any organ being involved, the lymph nodes draining that organ may also be affected and may indeed be an index of organ infection whether or not macroscopic lesions are present in that organ.

The main pathological features of the disease in the calf or young bovine can be summarised as follows:

(a) Primary infection (congenital or post-natal), characterised by its limitation to certain sites, by its early involvement of lymph nodes, and the early caseation and calcification; (b) local extensions of the caseous process; (c) scattered hæmatogenous disseminations to distant organs with the production of chronic progressive caseous nodular lesions; (d) acute miliary tuberculosis involving all lobes of the lungs and other organs; and (e) the acute process of multiple lobular caseous pneumonia. Although discussion has centred around the disease in the calf, primary infection and its varied sequels of dissemination can obviously occur at any age.

### Tuberculosis of the Adult Animal (Chronic Organ Tuberculosis)

The concept of chronic (isolated) organ tuberculosis of the cow, first brought forward by Nieberle, takes its origin from human standards. It will be recalled that, in the adult human being, chronic disease may remain restricted to the lungs for years or at the most only abortive lesions may form in extra-pulmonary tissues. This is in contrast with the extensive, and often fatal, hæmatogenous generalisation occurring after primary infection of the child. A period of years may elapse between the primary stage and the evolution of chronic phthisis and the latter is due to a reinfection of a resistant body from either external or internal sources. Nieberle has contended that a parallel chronic isolated process occurs only in the bovine species. In a previous section it was shown that there is no pathological counterpart in the dog and cat. The reason for this is not clear, but in those animals (and in the horse) the reaction of the tissues to the tubercle bacilli is atypical which may be an expression of a high degree of resistance even to a primary infection. It appears that the cow, of all animals, is the one most comparable to the human being in the sense that life is prolonged to an age in which the disease

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might be fully developed and one in which the rate of infection is similarly high.

The early development of the primary focus and generalisation in the child is well understood, but the early stages of the isolated lung disease of man have only been occasionally observed, and accidentally found, at autopsy because they are not usually fatal, and in a fatal case of advanced chronic phthisis a reconstruction of all previous pathological events is impossible. One of the first papers on the subject is that of Puhl (1922) who described the apical ("Reinfect") focus and its significance in the evolution of the disease. (Early apical foci and isolated pulmonary tuberculosis have, however, been experimentally produced in the rabbit by intravenous reinfections by Pagel [1936]—see Figs. 30 and 31.) The same difficulties arise with the chronic bovine disease and I have seen no clear description of early (apical?) foci in the cow which would correspond to Puhl's focus in man. In spite of the lack of morbid anatomical data which enable us to follow the events leading to chronic pulmonary tuberculosis in the cow, the pathological anatomy of the latter is distinctive and there can be little doubt we are dealing with a process similar to that seen in man. This is characterised mainly by the slow destruction of the lungs by caseation and liquefaction.

Chronic bovine tuberculosis resembles chronic phthisis in man (a type of chronic udder and uterus tuberculosis may come into the same category) in the following respects: (a) the predominating intra-canalicular manner of spread of infection (e.g., by the bronchial system in the lungs and by the ducts in the udder); (b) the absence or minor degree of calcification in the lesions; and (c) the absence, or abortive nature, of macroscopic lesions in the regional nodes—at least lesions corresponding in time to those in the organ, because calcified lesions may be present as relics of previous primary or disseminated infections.

The anatomical forms of the chronic pulmonary tuberculosis in the cow include the acinar and acino-nodular foci, the cavities, and the tubercular ulceration of the bronchi, trachea and appendicular organs. The acinus is the structural unit of the lungs; in the same way the acinar lesion can be regarded as the fundamental unit of the pathology of lung tuberculosis, and indicates clearly how infection is spread along the bronchial system. The lesions may be seen superficially, or on the cut surface, and are conspicuous in the way they delineate a lung lobule. The terminal bronchiole is filled with caseous material, and yellow caseous acini are clustered around to resemble the pattern of a clover-leaf. They may be localised in one area of the lungs, or widespread, and the centre of the acini may be liquefied and filled with pus while adjacent lung tissue may be compact and normal. The interlobular bronchi may have thickened walls and be ulcerated and also contain a semi-purulent detritus. The picture in general is reminiscent of that of catarrhal broncho-pneumonia. The foci may ultimately coalesce to form a large nodular consolidated area of

lung tissue. The basic histological picture shows confluent tubercles with lymphocytes, epithelioid and giant cells, and central caseation without calcification. As the small areas become confluent caseation is more marked although the elastic tissue may remain intact for some time and thus hold the caseated lung tissue together. Such destroyed lung tissue opens the way for secondary pyogenic infection, liquefaction and cavity formation.

The larger solid caseous areas of lung may become centrally liquefied and emptied into a main bronchus by coughing or the bronchial wall may be involved in the softening and undergo dilatation. The cavity is thus formed as a result of liquefaction and of bronchiectasis. In the cow small bronchiectatic cavitation predominates, in contrast to the large cavities produced in man by destruction of large areas of lung tissue, and are pathognomonic for chronic pulmonary disease. They are observed mainly in the dorso-caudal parts of the main lobe, have an open communication with the bronchial tree, contain yellowish-green purulent material, have hyperæmic margins and the adjacent lung tissue may be collapsed and indurated. The cavities may be tubular or saccular and may have obliterated vessels traversing across. It is destruction of bronchial wall, not initial dilatation, which forms the main feature.

Coughing and aspiration of infected sputum may result in implantation of organisms in the trachea, larynx and even tongue; the ulceration in these situations concomitant with chronic pulmonary disease is a common finding. The ulceration may be discrete or diffuse, irregular in shape, with raised borders and a yellowish-red base. The relatively common affection of the retropharyngeal lymph glands along with chronic pulmonary disease may be explained on the same grounds.

The regional lymph nodes of the lungs remain as a rule free from macroscopic changes, or, at the most, are uniformly swollen. The lesions seen in these nodes in chronic pulmonary disease—with marked calcification and fibrous encapsulation—are relics of previous infection and do not originate by spread from the lungs during this chronic phase. The pleura over affected parts of the lungs may be thickened, adherent to the costal pleura, or may be studded by small and large nodular proliferations, and a similar process may involve the pericardium.

The variety and combination of lesions which might be seen in the young and adult bovine lung may now be summarised.

(1) Encapsulated calcified foci due to the primary infection which may be single or multiple and accompanied by discrete disseminations in other parts of the lungs, and with corresponding calcified lesions in the regional lymph nodes.

(2) Irregular discrete scattered caseous foci of various sizes in one or both lungs indicative of bronchial erosion and spread by aspiration: a more even distribution might indicate an intra-vascular distribution within the pulmonary circulation.

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(3) Localised areas of caseous lung tissue made solid by the coalescence of the above.

(4) Acute caseous pneumonia in one lobe, or even a large part of one lung, in which the bacilli have been aspirated into the alveoli connected with the branching of an infected caseous or ulcerated bronchus.

(5) Profuse and wide dissemination of small tubercles, uniform in size, throughout all lobes, more numerous perhaps in dorso-caudal parts, and indicative of massive hæmatogenous infection causing acute miliary tuberculosis.

(6) Localised areas of caseous consolidation due to coalescence of myriads of miliary tubercles.

(7) Wide scattering through all lobes of irregular caseous foci much larger than the miliary tubercle and with a greater tendency to coalesce—multiple lobular caseous pneumonia.

(8) Acinar and acino-nodular lesions.

(9) Bronchiectasis and true cavities.

(10) Diffuse or nodular pleurisy.

Some of these are more commonly found in the disease of the young animal during the phase of primary infection; others in the chronic disease of the cow. The evolution, however, is not limited by age factors and an animal might be infected for the first time late in life. There is no reason to doubt that in some cases the course of events might then be similar to those occurring in the young animal, but we know that in other adult cows it can be remarkably rapid and one in which acute caseous pneumonia, caseous mastitis, caseous lymphadenitis, and miliary tuberculosis might predominate. These forms also occur in the adult animal as a phase of late generalisation in which a high degree of allergy may be present.

(Studies are still required similar to those carried out recently by Medlar (1940), who analysed the location of lesions in a large number of bovine lungs. He found that all areas of lung parenchyma were prone to be infected by single lesions (presumably this means primary infections), but that they were more numerous in dorso-caudal portions. The greater location of multiple lesions in the same area suggested a greater air-borne multiplicity of primary infection rather than spread within the lungs from a solitary focus. While chance was considered to play a part, he considered it could not account totally for a heavier seeding nor could the common belief of a more direct air flow be an explanation. In both men and cattle the uppermost portions of the lung lobes appear to be most susceptible to the disease (dorso-cephalic in man and dorso-caudal in cattle) and these are the portions which receive most direct air flow; he considers that posture may be a great influential factor.)

### Udder Tuberculosis

Tuberculosis in the mammary gland of the human being is a very rare finding in contrast to its high frequency in the cow for which there has been no adequate explanation. The constant "trauma" of lactation (high degree of physiological activity) may be an influential factor and is a usual explanation. In the human being chronic isolated organ tuberculosis is located exclusively to the lungs; according to Nieberle it is the most common form of the disease in the cow's udder.

There are three distinct pathological forms of tuberculosis of the udder, each of which has a different genesis. Some of the early papers describing the development of the udder tuberculosis almost certainly described these as various phases in the same process.

(1) *Acute Miliary Tuberculosis.*

This miliary process might occur during the same phase of early generalisation following primary infection when other organs may be frequently attacked. It is therefore an uncommon occurrence because at this stage the udder may not be fully developed. It represents the result of a heavy, bacillæmic invasion of the blood from some extramammary source with hæmic spread to the udder. As has already been pointed out, the regional lymph nodes of organs affected in this way are always involved. Small tubercles with central caseation and calcification may stud one quarter, or even the whole organ, and resemble histologically the tubercle described in an earlier part of the paper.

(2) *Caseous Tuberculous Mastitis.*

This form of the disease is to be regarded as part of early generalisation in a highly allergic body, or as developing in the adult upon failure of increased resistance. In the human being there are comparable examples in which the process is characterised by the development of extensive and rapid caseation. Large areas of the udder may be affected in this way, having an irregular, map-like distribution of soft, caseating, udder tissue bounded by a hyperæmic zone—giving rise to an appearance not unlike that of large infarcts. The supramammary lymph nodes are constantly enlarged and show caseous tuberculous lesions. Histologically, the lesion is distinctive and unmistakable, showing irregular bands of liquefactive necrosis, the margins of which are demarcated by an acute inflammatory reaction in which giant cells may be totally absent.

(3) *Chronic Udder Tuberculosis.*

According to Nieberle's findings this represents about 80 per cent. of all udder tuberculosis in the adult cow in Germany. The anatomical picture is totally unlike those already described and the typical caseous and calcified tubercle is not found. Macroscopically, the udder shows greyish-red intralobular foci with a lardaceous translucent appearance which may be replaced

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later by a hard confluent growth, reaching the size of a fist, in which fibrosis may be outstanding. Caseation is not marked, calcification is rarely present, and histologically the lesion consists essentially of a dense cellular infiltration composed mainly of large phagocytes. Spread of the disease throughout the udder occurs mainly by way of the ducts just as in the lungs it is mainly by way of the bronchi. The ducts may therefore be strikingly involved and have thick fibrous walls. The supramammary lymph nodes usually show no macroscopic changes except swelling and histologically only show follicular hyperplasia. This type is comparable to the chronic pulmonary disease due to reinfection, but whereas in the lungs exogenous sources appear to predominate, the udder must almost certainly be infected by recrudescence of internal lesions.

All the three forms described above are the result of blood-borne spread although debate has arisen regarding the chronic type. Ascending infection through the teat is anatomically possible, but must be regarded as highly unlikely. Retrograde infection of the udder from the regional lymph node or even the peritoneum has been suggested, but little or no supporting evidence can be produced to support this belief. It is unlikely that results can be obtained in investigations of this nature by ordinary post-mortem procedure in slaughterhouses. It might be advisable when such work is carried out for the udder to be examined by large sections, a method adopted for studying the spread of breast cancer in the human being and recently for the study of bovine udders in cases of streptococcal mastitis by Schalm and Haring (1939).

It is hoped that this publication, incomplete though it may be, will indicate sufficiently the lines of recent work and the problems that still await investigation. It is fully realised that omissions may be many, but this is unavoidable because of a consideration of space of publication. Much attention, for example, could with profit have been given to the special pathology of the disease.

In conclusion, I should like to quote a paragraph from a paper written by Ewart in 1882: "Of treatment and cure, which are the aims of all our labours, I have refrained from speaking, remembering that before we attempt to guide the forces of Nature we should clearly understand the ways in which they are put forth. But I venture to express a confident hope that the treatment of pulmonary cavities may look forward to a better future if it seeks its foundations in a *sound pathology*." In connection with animal tuberculosis we might ask ourselves whether these foundations have been assiduously determined.

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# JOHNE'S DISEASE INFECTION OF LABORATORY ANIMALS

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MOHLER (1939) reported the results of experiments in which he claimed that the injection into laboratory animals of cultures or tissues containing Johne's bacilli (*M. paratuberculosis*) emulsified in liquid paraffin gave rise to symptoms of disease within two or three weeks, followed usually by death in 30 to 90 days. For his experiments he used guinea-pigs, rabbits, rats, mice, pigeons and chickens and found that all but chickens proved to be readily susceptible, though these also developed lesions within a few months. The lesions were especially marked in guinea-pigs and rabbits and consisted of small greyish-white foci scattered over the serous membranes of the peritoneal and thoracic cavities with, in some cases, an extension under the capsule of the liver and other viscera. He also succeeded in carrying on the infection by injecting liquid paraffin emulsions and even saline suspensions of the affected tissues of guinea-pigs and rabbits into a further series of these animals.

He concluded that "in paraffin oil* we have a new tool or vehicle which, when mixed with *M. paratuberculosis,* is highly capable of infecting laboratory animals, either by reducing their resistance or by increasing the antigenic property or the virulence of the causal organism."

A series of experiments was carried out, using the technique described by Mohler.

### Experiment I

A strain of Johne's bacillus obtained from the Royal Veterinary College, London, was used. Cultures of the fourth generation, 2½ months old, grown on solid medium, were emulsified in liquid paraffin. A saline suspension of similar cultures was also prepared. Each of the inocula was injected intra-

* Paraffin oil is the term used in America to designate what in this country is termed liquid paraffin.

peritoneally into two rabbits, two guinea-pigs, two mice and two fowls. The rabbits, guinea-pigs and fowls received 4 mgm. of the culture and the mice 2 mgm.

*Saline Suspension.*

One guinea-pig died shortly after injection from an acute respiratory disease. All the other animals remained apparently healthy for 2½ months when they were killed and examined. One of the rabbits had a few small encapsulated caseous foci in the mesentery, smears from which showed a large number of acid-fast bacilli. Cultures, however, yielded only one or two colonies, indicating that the majority of the organisms were dead. Similar nodules were seen in the guinea-pigs. None of the other animals showed any lesions.

*Liquid Paraffin Emulsion.*

All the injected animals remained healthy for 2-2½ months when they were killed and examined. Lesions were found in all the animals.

*Rabbits.*—In the mesentery near its attachment to the intestine, caseous nodules the size of a pea were found, as well as hard, whitish nodules superficially in the liver, varying from the size of a pin head to a pea.

*Guinea-pigs.*—The spleen was enlarged and encased in the enormously thickened omentum, the whole forming a large mass firmly adherent to the abdominal wall. Small, hard, yellowish-white nodules were present on the abdominal surface of the diaphragm and greyish-white, sharply circumscribed lesions in the liver. Most of these were superficial but some penetrated more deeply into the liver substance. In addition, there were present diffuse hard thickenings ramifying more or less deeply into the substance of the liver at its thickest part.

*Mice.*—There were numerous small whitish nodules throughout the lining of the abdominal cavity so closely arranged as to form thick whitish masses. A few nodules were also present on the surface of the liver, but the spleen was but little affected.

*Fowls.* — Numerous pinpoint nodules were present throughout the mesentery. A few nodules were also seen in the liver which was covered with a greyish-white exudate. The nodules in the liver were, on the whole, smaller and more superficial than in either rabbits or guinea-pigs.

*Passage Experiments.*

Parts of the affected mesentery and liver of one of the rabbits were ground up in saline and injected intraperitoneally into a rabbit, two guinea-pigs, two mice and two fowls. Smears from the emulsion showed a few acid-fast bacilli. The mice died in one and two months respectively, but on post-mortem examination showed no lesions. One fowl died of extreme emaciation 11 days after inoculation, but showed no recognisable changes in the internal organs. The rabbit, two guinea-pigs and the remaining

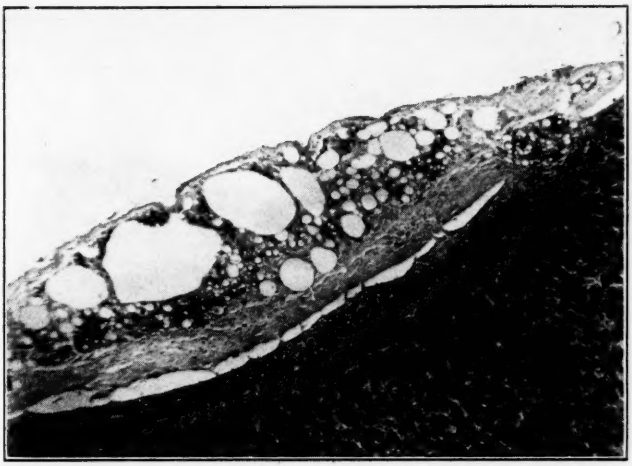

FIG. 1.—Section of nodule from liver of a fowl caused by the intraperitoneal inoculation of liquid paraffin emulsion of Johne's bacilli. The nodule consists of a network of connective tissue in which are numerous rounded vacuoles. Underlying is an intermediate zone infiltrated with lymphocytes, histiocytes and a few giant cells. X 65.

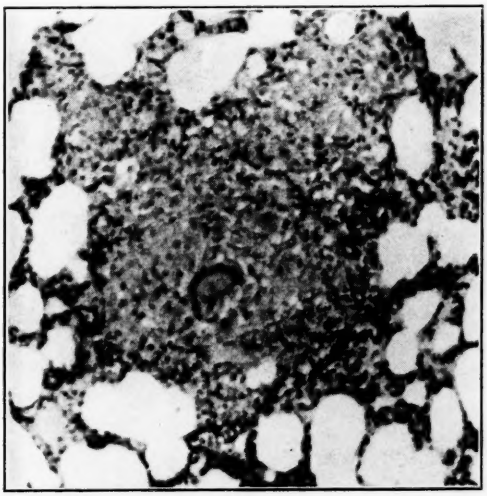

FIG. 2.—Section through a small pearly tubercle in the lung of a guinea-pig inoculated intraperitoneally with liquid paraffin emulsion of B. phlei. Note the epithelioid cells and a giant cell. X 125.

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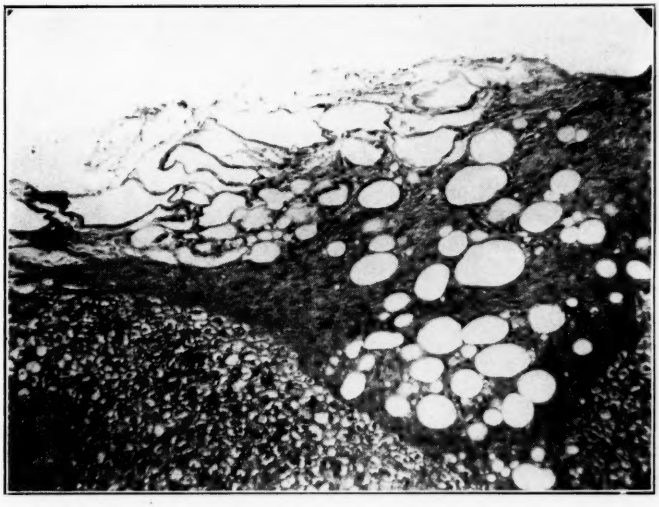

FIG. 3.—Nodule in the liver of a rabbit inoculated with liquid paraffin emulsion of Johne's bacilli. Note the more deep-seated and infiltrating character of the lesion. X 65.

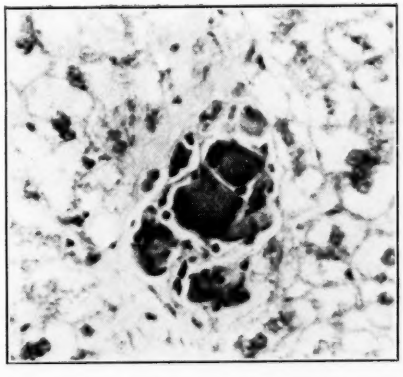

FIG. 4.—Focal lesion in the liver of above rabbit. It consists almost entirely of giant cells with a few round cells. X 300.

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fowl were killed three months after inoculation, but except for the presence of three hard whitish nodules on the surface of the liver of one guinea-pig, no lesions were seen in any of the animals. Smears from the guinea-pig lesions showed only rare acid-fact bacilli and cultures yielded no growth.

Similarly, nodules from the affected guinea-pigs were ground up in saline and injected intraperitoneally into two guinea-pigs. They remained healthy and were killed after three months. One showed no lesions. In the other, a few nodules containing caseous pus fairly rich in acid-fast bacilli were found in the mesentery. Cultures failed to yield any growth.

**Experiment II**

The same strain of the organism was used as in Experiment I, the culture being the fifth generation grown on solid medium for three months. A liquid paraffin emulsion only was used, two rabbits, four guinea-pigs and six mice being injected intraperitoneally, the rabbits and guinea-pigs receiving 8 mgm. culture (2 c.c. emulsion) and the mice 4 mgm. (1 c.c. emulsion).

*Rabbits.*—They remained healthy and when killed after 3½ months showed no lesions, but there is some doubt concerning the injection having been made intraperitoneally.

*Guinea-pigs.*—Two died from intercurrent disease, 19 and 60 days respectively, after injection. In both, lesions similar to those described in Experiment I were found. The other two guinea-pigs remained healthy and were killed after 3½ months. Both showed ascites and the usual lesions in the internal organs. The portal glands were enlarged and in one guinea-pig enlargement of the sternal glands was also found. Smears from the portal and sternal glands showed a few acid-fast bacilli. Cultures from the lesions yielded no growth.

*Mice.*—These were killed after 3½ months and showed lesions as described in Experiment I. The lesions were very rich in bacilli, yielding numerous colonies in culture. The number of colonies, however, was considerably less than the number of organisms seen in smears. This suggests that the bacilli were in the process of being destroyed and that no actual multiplication had taken place.

*Passage experiments.*—Nodules from the two guinea-pigs killed after 3½ months were emulsified in liquid paraffin and others suspended in saline. Each of the two materials was injected intraperitoneally into two guineas-pigs. Smear examination showed the emulsions to be very rich in bacilli, but cultures, as was found subsequently, yielded no growth.

*Saline suspension.*—The guinea-pigs were killed after 3½ months. In the omentum of both, three or four encapsulated caseous foci were found. A few acid-fast bacilli were seen in smears but cultures yielded no growth.

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*Paraffin emulsion.*—One guinea-pig died of acute respiratory disease 26 days after being injected. One small nodule was found in the liver. The other died from the same disease after 40 days. No lesions were found.

**Experiment III**

For this experiment Johne's bacillus, Weybridge Strain 2, was used. It was grown on solid medium for 69 days. Part of the culture was emulsified in liquid paraffin, and part was killed (heating at 65 deg. C. for 30 minutes and proved killed by culture test) and emulsified in liquid paraffin.

*Dead culture.*—Two guinea-pigs were injected intraperitoneally, one receiving 7 mgm. and the other 0.7 mgm. The former died 36 days after injection. Lesions similar to those produced by the live organisms were present, though they did not seem sufficiently extensive to have caused the death of the animal. The other guinea-pig was killed two months after injection. It had shown no symptoms of illness. Lesions of the usual type were found.

*Live culture.*—Two guinea-pigs received intraperitoneally 0.7 mgm. and 14 mgm. of the culture, respectively. The former died after 50 days. Slight lesions only were found. The other guinea-pig remained healthy and was killed after two months. Extensive lesions were found in the abdominal organs and the sternal glands were involved.

**Experiment IV**

A culture of Johne's bacillus isolated by the writer from a Guernsey cow was used.

An undetermined dose of a liquid paraffin emulsion prepared from a second generation culture, 52 days old, was injected into two guinea-pigs. Both remained healthy and were killed in two months. Lesions of the usual type were found.

**Experiment V**

This experiment was carried out to ascertain whether similar lesions would follow the intraperitoneal injection of liquid paraffin emulsions of acid-fast organisms other than Johne's bacillus. A human strain of the tubercle bacillus and B. phlei were used.

*Human Strain of Tubercle Bacillus.*

A liquid paraffin emulsion was prepared as above from killed cultures and a saline suspension of dead culture was also made. Two guinea-pigs were injected intraperitoneally in each case. The doses were 1 mgm. and 4 mgm. respectively.

*Saline suspension.*—The guinea-pigs remained in good health and were killed after five weeks. In both, a few greyish foci were found on the surface

of the liver and in one of the animals there were a few encapsulated caseous foci in the omentum, very rich in acid-fast bacilli.

*Liquid paraffin emulsion.*—The guinea-pigs were killed after five weeks, during which time they had remained apparently healthy. In both were found extensive lesions similar to those seen in guinea-pigs treated with emulsions of live or dead Johne's bacilli. In addition, numerous pearly tubercles were found in the lungs. These lesions were not seen in guinea-pigs treated with Johne's bacilli.

*B. phlei.*

Using moist 17-day-old cultures from the surface of glycerine broth, emulsions of living and dead organisms were made in liquid paraffin and a suspension of living organisms in saline.

*Liquid paraffin emulsion of living organisms.*—Two guinea-pigs were injected intraperitoneally, one receiving 4 mgm. and the other 40 mgm. The former died after a month. Lesions were found in the abdomen. The omentum was thickened and completely folded over the spleen. The intestines, liver, etc., were adherent to the diaphragm and the abdominal wall. The adhesions, when cut, showed collections of caseous yellow pus which contained large numbers of viable acid-fast organisms. In the lungs were small glassy foci. The other guinea-pig (which received 40 mgm.) died in 20 days and similar lesions were found. The lesions in these guinea-pigs were, on the whole, more extensive than those seen in guinea-pigs injected with Johne's bacilli.

*Liquid paraffin emulsion of dead organisms.*—Two guinea-pigs received intraperitoneal injections of 75 and 10 mgm. respectively. The former died in 27 days when lesions were found similar to those in guinea-pigs which had received living culture. Nodules were found on the liver and diaphragm, the sternal glands were enlarged, there was fibrinous peritonitis and in the lungs were numerous glassy tubercles. The other animal was killed after 47 days when lesions of a similar type were found, though somewhat less extensive.

*Saline suspension of living organisms.*—Two guinea-pigs received intraperitoneally 5 and 50 mgm. respectively of the living culture suspended in saline. Neither showed any symptom of ill-health nor were any lesions found when they were killed after two months.

**Experiment VI**

As a control to the above experiments, 1 c.c. liquid paraffin was injected intraperitoneally into each of two guinea-pigs. One died of an acute respiratory disease after six days. Unabsorbed liquid paraffin was found in the abdominal cavity. The other guinea-pig was in good health when killed after 3½ months. No lesions were found. In the abdominal cavity were a few globules of unabsorbed liquid paraffin.

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### Histology of the Lesions

The hard granulomatous lesions produced by inoculation of liquid paraffin emulsions of Johne's bacilli have an essential identity of structure in all animals irrespective of the organ in which they are found. They are indicative of the resistance to infection with this organism possessed by laboratory animals, and consist of connective tissue in which are numerous vacuoles and which is infiltrated to a varying extent with lymphocytes and histiocytes. A few blood vessels are also usually present.

The lesion is generally a superficial one, especially in the fowl (Fig. 1), and is connected to the underlying healthy tissue by a narrow intermediate zone in which can be seen large numbers of lymphocytes, histiocytes, a few giant cells of the Langhans type and broken-up parenchymatous cells. Occasionally bits of parenchymatous tissue may be entirely cut off and found within the nodule enclosed in a fibrous capsule intermingled with other cellular elements.

In the rabbit and guinea-pig the changes are more deep-seated and infiltrating, resulting in greater damage to the parenchymatous elements and their replacement by fibrous tissue (Fig. 3). Occasionally in the liver one sees numerous small foci consisting of collections of epithelioid cells, histiocytes and a few giant cells.

Sections of the liver of one rabbit revealed a few focal lesions composed almost exclusively of giant cells with but a few lymphocytes (Fig. 4). Lesions of this type have been described by Mathews (1930) in cattle and are believed by him to be of metastatic origin.

The small translucent tubercles seen in the lungs of guinea-pigs following intraperitoneal inoculation of liquid paraffin emulsions of live or dead B. phlei and dead human tubercle bacilli are composed mainly of epithelioid cells with a few giant cells (Fig. 2).

### Discussion

No difficulty was experienced in setting up lesions similar to those described by Mohler by the intraperitoneal injection of liquid paraffin emulsions of Johne's bacilli into guinea-pigs, rabbits, mice and fowls. In the guinea-pig the omentum invariably reacted and became thickened and folded over the spleen, the whole forming a compact mass adherent to the abdominal wall. Hard, whitish nodules were also found on the surface of the liver and, in some cases, they penetrated deeply into the liver substance. In all injected guinea-pigs hard, yellowish-white thickenings were found on the abdominal surface of the diaphragm. Lymphatic glands, especially the portal and, in some cases, the sternal, were involved. Scattered foci were found in some cases in other parts of the abdominal cavity.

In rabbits the distribution of the lesions was somewhat similar, but the

lesions were found mostly in the liver. A few nodules were present in the mesentery and sometimes in other parts of the abdominal cavity.

In mice the lesions were confined almost exclusively to the serous membrane which in places became so thickly studded that soft greyish-white masses were formed. The liver and spleen were usually free.

In fowls the lesions were usually small, hard and whitish and were present mostly on the serous membrane. A few lesions were found on the surface of the liver.

These results confirm some of the findings reported by Mohler. Further experiments, however, show that the lesions in the guinea-pig are not specific for Johne's bacillus, as similar lesions, though perhaps varying in intensity, can be set up by the injection of liquid paraffin emulsions of live and dead B. phlei, dead human tubercle bacilli and even dead Johne's bacilli. A point of difference in the lesions set up by dead human tubercle bacilli and live and dead B. phlei is the occurrence of small translucent tubercles in the lungs; they were not seen when Johne's bacilli were used. Hagan and Levine (1932), however, have described such lesions following the intraperitoneal, intramuscular and even subcutaneous injection of a wide variety of acid-fast organisms and it seems likely that their formation is dependent on the fineness and diffusibility of the injected emulsion rather than their being specific reactions caused by any particular acid-fast organism.

That the lesions are dependent upon the presence of both the organisms and the liquid paraffin is shown by the fact that liquid paraffin alone causes no lesions, though globules of unabsorbed oil may be detected in the peritoneal cavity for months afterwards and that saline suspensions of the organism alone cause only minimal lesions, mostly confined to the omentum and the liver. The omentum is particularly prone to react and similar lesions in the experiments above described have been shown to be caused by dead Johne's bacilli and even dead human tubercle bacilli.

The results of these experiments, however, do not confirm Mohler's findings that guinea-pigs usually die within a few weeks after the intraperitoneal injection of Johne's bacilli suspended in liquid paraffin. The majority of the guinea-pigs used in these experiments appeared to be healthy when killed (in most cases 3½ months after the injection); any deaths, except perhaps in one or two cases, were due to intercurrent infection. It was remarkable that the animals showed no symptoms of ill-health in spite of the extensive lesions in the abdominal cavity. Some of the guinea-pigs inoculated with live or dead B. phlei died, but these had received comparatively large doses.

According to Mohler, mice are apparently quite susceptible to Johne's bacillus and are stated to die in 9-24 days after inoculation. The mice used

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in these experiments were kept under observation for periods up to 3½ months without showing any evidence of ill-health, though extensively diseased.

It is also evident from these experiments that the reaction in the abdominal cavity is not dependent on the multiplication of the injected organisms. When live cultures were used the number of viable organisms recovered from the lesions after 3½ months was very sparse and the same type of lesions occurred when killed cultures were used.

A further point of difference between the results of these experiments and those carried out by Mohler concerns the transmission of Johne's bacillus in series through guinea-pigs and rabbits. These experiments gave negative results.

It appears to the writer that such differences as he has noted between the results of his work and those obtained by Mohler may be attributable to the age of the cultures used in these experiments. This point is now being tested.

## Conclusions

Considering all the above results it would appear that the lesions set up by the intraperitoneal injection of liquid paraffin emulsions of Johne's bacilli are not specific. They have been produced by other types of acid-fast bacilli, whether living or dead. Multiplication of the organisms does not take place in the abdominal cavity and inoculated animals, though extensively diseased, may continue to live for prolonged periods. When death occurs it is not due to active disease set up by the organisms, but rather to functional interference caused by the lesions, the extent of which seems to depend on the dose injected. Similar fatal results can be produced by other acid-fast organisms, living or dead, including saprophytic organisms.

## Acknowledgments

The writer is deeply grateful to Professor T. Dalling, Director, for the facilities accorded for carrying out this work and for his interest and advice at all times. He is also indebted to Mr. W. J. Smith, of the Institute, for the preparation of the micro-photographs.

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# THE RESULTS OF THE INOCULATION OF GUINEA-PIGS WITH M. PARATUBERCULOSIS (Ovine Type) IN LIQUID PARAFFIN

By A. WILSON TAYLOR
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THE effect of the inoculation of liquid paraffin emulsions of 14 types of saprophytic acid-fast bacilli and killed cultures of *M. paratuberculosis* and of both bovine and avian types of *M. tuberculosis* in guinea-pigs was studied by Hagan and Levine (1932). Following intraperitoneal inoculation, lesions in various stages of fibrosis were found in the abdominal cavity, while tubercles which in many instances had undergone caseation were present in the lungs. Three guinea-pigs inoculated by these authors with heat-killed *M. paratuberculosis* (Johne's bacillus) and destroyed at the end of a period of 68 days all showed lung lesions. Mohler (1939) demonstrated a similar condition in guinea-pigs subsequent to the intraperitoneal inoculation of heavy suspensions of living M. *paratuberculosis* in liquid paraffin. In this instance, death occurred after periods varying from 7 to 32 days with the formation of nodules in the abdominal and thoracic viscera, and enlargement of the lymph nodes.

These workers used cultures of *M. paratuberculosis* of bovine origin in the preparation of their inocula. It has been suggested by Dunkin (1936) and also by McEwan (1939) that the acid-fast organisms found in cases of so-called Johne's disease of sheep may not be identical with those found in the bovine since considerable difficulty is experienced in obtaining cultures of the ovine type. Dunkin and Balfour Jones (1935) obtained growth only after an incubation period of seven months, while McEwan (1939), using various media, was unable to cultivate the organism. In the experience of the writer, a scarcely discernible growth occurs after seven to eight months' incubation.

Since satisfactory cultures were not available, it was necessary, in order to repeat Mohler's experiments with an ovine strain, to use organisms obtained by treating affected sheep's bowel with antiformin. By emulsifying these in liquid paraffin and inoculating guinea-pigs intraperitoneally, results comparable with those of Mohler were obtained.

## Methods

The mucous membrane of part of the small intestine of a five-year-old ewe affected with Johne's disease was macerated in 15 per cent. antiformin for one hour at 37 deg. C., filtered through very fine wire gauze and centrifuged. The sediment was washed three times in sterile normal saline and on examination was found to be very rich in acid-fast organisms. Approximately

2.0 g. were added to 22.0 c.c. of sterile (autoclaved) liquid paraffin and an even milky white suspension obtained by rubbing in a mortar. Approximately 0.25 g. of sediment was shaken up in sterile normal saline solution and corresponded in density to No. 6 on Brown's scale. Ten healthy male guinea-pigs, between 450 and 550 g. in weight, were inoculated intraperitoneally with 2.0 c.c. of the oil suspension, while, as controls, three guinea-pigs were inoculated similarly with 2.0 c.c. of the saline suspension and two with 2.0 c.c. of liquid paraffin only.

Fourteen days after inoculation several of the guinea-pigs inoculated with the oil suspension showed ruffled coats, did not eat and lost weight rapidly. Eventually eight of the animals showed such symptoms and died from 15 to 31 days after inoculation. The lesions in these animals are described below.

The remaining two animals, and the five controls, died from the 34th to the 37th day after inoculation during a period of intense frost. A number of normal guinea-pigs maintained under similar circumstances died at the same time. No lesions were found in these animals.

The lesions were essentially similar in all animals that showed symptoms. In the abdominal cavity, the peritoneal ligaments, the omentum and, in parts, the mesentery, were greatly thickened, white and fibrous. The spleen was enlarged to approximately twice its normal size and was in many cases adherent to the abdominal wall. White nodules, from a pin head to a rice grain in size, were usually present on the surface of the liver and spleen. These could be completely removed with forceps, leaving a small pit in the tissue. No nodules were at any time detected in the substance of any organ. White cartilage-like plaques were present on the surface of the diaphragm, but enlargement of the lymph nodes was not observed. Throughout the peritoneal cavity minute droplets of oil, presumably liquid paraffin, were detected. In the thorax, nodular lesions were observed in the lungs of two guinea-pigs. Unfortunately, these were prematurely discarded, and were therefore not examined further.

Microscopically, the structure of the nodules on the liver and spleen, and the thickened areas on the peritoneum, appeared similar. The lesions consisted of a network of fibrous tissue and capillaries, intermingled with cellular elements comprising mainly histiocytes and large and small lymphocytes. Vacuoles which occurred throughout the lesion probably marked the positions previously occupied by oil globules. A few polymorphs were observed, but no giant cells or plasma cells were seen (Fig. 1). Occasionally a small mass of homogeneous, probably caseated, material was evident. Organisms, singly or in clumps were scattered throughout the lesions, but predominated in the more cellular areas. Masses of organisms were also seen intracellularly, and here resembled the closely packed organisms seen in the bowel in natural cases of Johne's disease. The spleen pulp appeared congested, large numbers of small lymphocytes were present, among which giant cells of the Langhans type

FIG. 1.—Thickened Peritoneum. H. & E X 450.

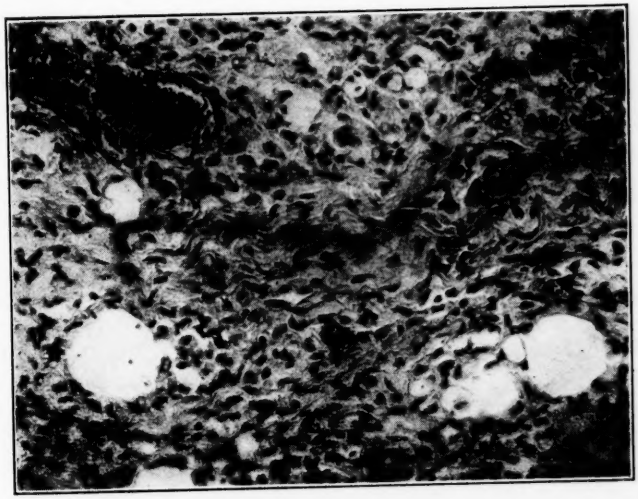

FIG. 2.—Liver. H. & E. X 400.

were a constant feature. The liver was congested, and Glisson's capsule was infiltrated with endothelioid cells and lymphocytes. Giant cells were frequently present, and occasionally single acid-fast organisms, of similar morphology to *M. paratuberculosis*, were found in association with the lesion (Fig. 2). This phenomenon would appear to be similar to that described by Mathews (1930) in the liver of cattle affected with Johne's disease. Hallman and Witter (1933) also found focal lesions in the liver of affected cattle, but were unable to associate them with acid-fast bacilli.

No attempt was made to secure cultures from any of the lesions.

The experiments recorded above would appear to confirm the observations of Mohler (1939) that *M. paratuberculosis* when suspended in liquid paraffin is capable of producing a fatal disease in guinea-pigs. It is unlikely that minor differences between his results and those recorded here can be traced to the use of the ovine type of *M. paratuberculosis*, as Hagan and Levine (1932) have shown that both saprophytic and heat killed pathogenic acid-fast bacilli produce a similar effect.

**Summary**

The intraperitoneal inoculation of ten guinea-pigs with a liquid paraffin suspension of *M. paratuberculosis* of ovine type resulted in the death of eight within 31 days. Granulomatous lesions in the peritoneal cavity are described, and the presence of lesions associated with acid-fast bacilli in the liver is noted.

The writer is indebted to Dr. H. H. Holman for his advice on the pathology of the conditions described, to Mr. George R. Knight for the microphotographs, and to the Director of this Institute, Dr. J. Russell Greig, for his continued interest in this work.

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# CORYNEBACTERIUM EQUI IN BOVINE PYOMETRA

**By J. F. CRAIG and G. O. DAVIES**

*Department of Veterinary Pathology, University of Liverpool.*

USUALLY it has been considered that when organisms of the genus Corynebacterium are found in connection with bovine affections they belong to the species *Corynebacterium pyogenes.* That this is not so always is demonstrated in an outbreak of bovine sterility which came under our observation in April,

1940. In this outbreak 20 dairy cows in a herd of 50 were not in-calf and six abortions at three or four months had occurred during the preceding six months. Following upon the abortions there was a little temporary vaginal discharge. Two of the cows were found to be affected with pyometra and samples of the uterine discharges were obtained for examination. The cows gave no reaction to the agglutination test for *Brucella abortus* infection.

The discharges were semi-fluid, yellowish-grey and opaque. In them no Trichomonads could be found, but in films there were numerous leucocytes, catarrhal cells and organisms like Corynebacteria. These organisms were isolated in pure culture and in many of their characters, but not all, resembled *Corynebacterium equi.*

*Morphology.*—The organisms appeared as pleomorphic rods, some of which were curved or Y-shaped. Some were thickened at one end. In 24-hour cultures some of the organisms formed chains, and branching was noted, while a few days later coccus or ovoid forms appeared which sometimes formed chains. They were gram-positive, but only sometimes was the staining irregular. They were non-motile, non-sporing and aerobic.

*Cultural Characters.*—The optimum temperature for growth was 37 deg. C., but the organisms showed slight growth also at room temperature.

*Agar.*—Growth appeared in 24 hours at 37 deg. C. Small round dry raised colonies appeared which were white and opaque and tended to fuse rapidly, forming a uniform layer which became salmon-pink in colour in the course of a few days. The growth was not moist, but glistening in parts and not exactly dry. With further subcultivation the growth assumed a wrinkled appearance.

*Blood Agar.*—Good growth which became pink in two or three days. There was no liquefaction.

*Potato.*—A salmon pink scanty growth in three days.

*Broth.*—A granular growth at the bottom and a thin dry layer on the surface. No turbidity of the medium.

*Gelatin Stab.*—Slight growth along the tract of the needle and on the surface. No liquefaction occurred.

*Biochemical Tests.*—No changes were observed in glucose, lactose, saccharose, maltose, salicin, inosite, arabinose or mannite. Litmus milk remained unchanged. Indol was not formed and the organism was negative to methyl red and Vosges Proskauer tests.

In its growth and reactions this strain has been compared with two strains of *C. equi* which were kindly forwarded by Dr. Lovell and had originally been obtained from Magnusson. They were from the pig and foal respectively. The only differences between these strains and our bovine strain were that the bovine organism on solid media gave a drier growth and produced no turbidity in broth.

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Bruner, Dimock and Edwards (1939) have demonstrated by serological tests that *C. equi* possesses both species-specific and type-specific antigens. No attempt has been made by us to confirm the value of these tests in the identification of the organism isolated from the cases of pyometra, but from the observations otherwise made it may be taken that the differences between our bovine organisms and *C. equi* are so slight that they may be regarded as variants of the same species. If that be admitted, then the organism we have isolated from the cow is the first example of *C. equi* isolated from the bovine.

Up to the present *C. equi* has been demonstrated only in pulmonary abscesses and other lesions of pyæmia in foals by Magnusson (1938), Bull (1924) and others, in the os uteri of barren mares and aborted equine fœtuses by Bruner, Dimock and Edwards (1939), in the cervical glands of the pig with lesions slightly resembling those of tuberculosis by Magnusson (1938), Bendixen and Jepsen (quoted by Magnusson) and Plum (1940), and in the uterine discharge of a she-buffalo following abortion by Rajagopalan and Gopalakrishnan (1938).

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# QUITTOR

## By HARRY LOMAS, M.R.C.V.S.

THIS affection, sited on the coronet at the hinder part, is regarded by many as quite a protracted condition and one that involves many disappointments in the course of treatment.

This must have been so, or the surgical method of incising under the coronary band would not have had so much vogue as it did. When the necrosed area was exposed it was seen to be small, irregularly shaped, and perhaps no larger than one's little finger nail; yet it looked very pretty in its grass-green colour lying over the cartilage as one saw it when the horse was under chloroform and the tourniquet was in position.

There was small wonder that the operation went into disuse, since the wound took too long a time to heal and there was often so much labour in dressing, etc., as to render it commercially unsound. There often remained the permanent fault of false quarter as a direct result of the operation, and the horse was not so good in value as before. Chemical methods have been

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in use against quittor for a very long time, mercuric chloride being one of the principal agents; it was applied on a thin wisp of damp tow or even rolled up in thin paper and inserted into the sinus.

There was much sloughing and many failures, most likely because the chemical failed to reach the little necrotic flake. This dead portion is always super-imposed upon the outer side of the cartilage and it is usually the result of a slough (bacillary necrosis) or an abscess from any cause in that locality.

Sloughs, whether due to treads or bacillary necrosis, are the most frequent cause of quittor. And any acute inflammatory condition locally should be reduced before a potent injection such as will be employed for quittor is given, the object being to prevent needless sloughing.

The inference is that if a chemical injection could be successful sometimes, then there must be a good reason for the failures, whether the obstacle intervening is simple or difficult.

It was the surgical operation that taught the writer the way to success by passing probes along the sinus when the horse was on the bed and before the necrosed tissue was removed. It was sometimes found that a stiff probe would not reach the part by reason of a curve or bend, whereas a flexible one could be adjusted to overcome this hindrance.

To this end, two little ideas were brought into use, one being flexible metal pipes of different lengths for the syringe, the other being gum-elastic tubes with a calibre about the same as that of an ordinary quittor pipe, but having one end slightly funnel-shaped in order to receive a stiff pipe for the injection.

The lengths of these tubes are one, one and a half, two, two and a half, and three inches.

It was found to have no purpose to increase the length of any pipe; further, it became convenient to have similar lengths of all the three varieties.

The gum-elastic tube has one advantage in a difficult case in that it can be inserted and left in the sinus for a minute or so in order to soften by reason of the body heat, and then be gently pushed to the end so that the injection can be given.

The size of the syringe is entirely a matter of personal liking; the writer prefers one of ½-oz. capacity as it allows one to get nearer to the work.

A solvent which does not cause sloughing of the skin would be preferred in commercial practice since time lost is so much money lost, and the following prescription is one that fulfils that object and has done good service for about 30 years:

| | | | | | | |
|---|---|---|---|---|---|---|
| Hydrarg. Perchlor. | ... | ... | ... | 1 | dram |
| Acid Acetic Glac. ... | ... | ... | ... | 1½ | „ |
| Aqua ... | ... | ... | ... | 16 | „ |

Directions: The Hydrarg. Perchlor. must be dissolved in boiling water and the Acid Acet. Glac. poured in just after the receptacle is taken away from the heat.

It cannot be overstated that it is not wise to inject when the foot is on the ground, taking all the weight of the leg upon it, with the liability of compressing the sinus. For preference, have it put on a tripod or on the farrier's knee.

Injections are given at intervals of two days, though one may suffice in the case of a short, straight sinus: but if two do not give satisfaction, it becomes a case for the flexible metal or gum-elastic pipes. After injection, on examination of the cotton-wool dressing with which it has been bandaged, one should find a muddy looking discharge, in which, if the case is cured, there will be a mass, pinkish-grey in colour, which can be cut by the thumb nail. This is the remains of the former bit of necrosed cartilage which has been altered by the chemicals.

There is very little more now to do to the case except to keep the parts aseptic, and in a fortnight it will not be easy to find the orifice of the sinus.

This method of treatment of quittor is one which is efficient and has stood the test of being a sound commercial proposition.

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# BOTRYOMYCOSIS—SHOULDER TUMOUR IN HORSES

By HARRY LOMAS, M.R.C.V.S.

To those who have to do with large studs, this affection is sufficiently frequent, and often so intractable as to cause considerable loss of service. Surgical methods do not always produce the happy results in the end which one sought to achieve.

The most usual situation for the tumour is about two or three inches from the point of the shoulder, and most commonly there is mere tumefaction without any sign of abscess; yet one does occasionally receive a case which shows early and definite abscess formation. The writer regards the latter as being a fortunate circumstance, since it will provide the material for taking a swab from which a vaccine may be prepared.

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The swab should be sent to a bacteriologist, and if the report upon the resulting culture describes it as a pure one of a staphylococcus resembling the botryomyces, then one may regard it as satisfactory. The vaccine may be used, not only on the horse from which it was derived, but also on any other cases, present and future, until the supply is exhausted.

In the times when horses, and therefore cases, were more prevalent, it would not be long before there was another case, giving hopes of another available supply of vaccine; but failing this, there was always the possibility of borrowing a case from another infirmary. However, of late it has not been so easy to carry out this method by reason of the reduction in the size of studs.

*Treatment by Vaccine.*—Vaccines have been prepared at the Royal Veterinary College. The dose is one c.c. and it is injected under the skin of the breast over the pectoral muscles after thoroughly asepticising the parts; six days later a dose is injected into the opposite side; while for the third and last dose one reverts to the side where the first injection was made, choosing a spot which was not the exact site of the former injection, as there may be a little swelling remaining.

There is always a local reaction, varying in size from two inches by one to five or six by three, but these variations do not seem to have any significance whatever, and they have very often dispersed entirely by the fifth day.

Having given these injections, one begins to look for signs of retrogression in the size of the tumefaction about the sixteenth day, and it is quite pleasing and remarkable to see the gradual subsidence that takes place, until from the 26th to the 32nd day there is practically no enlargement remaining, and the case can be discharged to resume work.

It is said that the exception proves the rule, and there is one, or rather two, in connection with this treatment. For instance, occasionally an abscess will mature during the process of treatment and it may be necessary to apply surgical measures. The latter will delay the resumption of work, but does not interfere with the cure. The other exception has regard to the matter of sub-cultures. In the past, when the primary vaccine was exhausted, one naturally applied for a further supply to be sub-cultured from the original which had done such good service. The product of sub-culture never gave the same results—they were slower in reducing the tumour, or did not completely reduce it, and there were recurrences. Yet, when it was possible to make use of a primary culture on these recurrences, quite satisfactory results were obtained and in about the same period of time.

This method of treatment has been in use since the year 1921; scores of cases have been treated, and with the exceptions noted, have remained free of this affection during their working life in London.

I am indebted to Dr. F. C. Minett, of the Royal Veterinary College, for his assistance and for the preparation of the vaccines.

# Review

**A Guide to Human Parasitology.** By D. B. Blacklock, M.D., D.P.H., D.T.M., and T. Southwell, D.Sc., Ph.D. Fourth Edition. Pp. viii + 260. 122 Illustrations and 2 Coloured Plates. London: H. K. Lewis & Co., Ltd. Price 12s. 6d. net.

A fourth edition of this book will be welcomed by all those who have, since its first publication in 1931, learned to rely upon it. Written for readers who have had no experience of human parasitology and for medical practitioners, it gives them a sound and practical introduction to the subject and will be valuable also to students of the parasites of animals other than man. It is well illustrated by plenty of excellent line drawings and two coloured plates, one of the malarial parasites, the other of the cysts of *Giardia, Entamœba histolytica* and some other human intestinal amœbæ as they appear when they are stained with iodine. Both plates are valuable, but the reader should realise that he will hardly see in his smears malarial parasites quite so vividly coloured, nor will the rings of *Plasmodium falciparum* appear so thick and obvious as they are shown in the plate. The amœbic cysts are also rather too vividly depicted. Another valuable illustration is the one which shows the eggs of the commoner helminths drawn to the same scale so that their sizes can be compared.

The text requires little comment. A small oversight is that on page 107 Cysticercus bovis and C. cellulosæ should be in italics. A useful chapter describes the use and care of the microscope and how to find objects, focus them and measure their size. Excellent advice about sizes is given on page 2, where the authors emphasise that too much importance is often given to the sizes of parasites and that great variations of size must be expected within the individual species of animals because they are living things. The sections on technique are good, although there is no mention of lactophenol as a clearing agent for nematodes, carbolic acid alone being recommended by the authors for this purpose. Salt is recommended for the floatation of nematode eggs and no mention is made of Sheather's saturated sugar solution which is less likely than salt to crumple eggs by osmotic effects; if sugar rationing prevents its use, 30 per cent. glucose may be substituted.

The tables at the end of the book help the reader to understand broad points, such as the parts played by water and intermediate hosts in the spread of parasites, the distribution of parasites among the tissues of the body and their geographical distribution. Their life histories are also diagramatically shown. Chapter 22, with its brief accounts of myiasis, congenital infection through the placenta, post-mortem examination and the so-called immunity

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to parasitic infections, is a useful one, although justice can hardly be done in so small a space to the last-named problem, about which we have yet to learn facts which may have an important bearing on theories of immunity in general. The publishers are to be congratulated on their production of this valuable book.

G.L.

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## CORRIGENDUM

In the article on "A Case of Cyclopia in the Foal," by H. V. Hughes, B.V.Sc., M.R.C.V.S., D.V.H., and J. W. Dransfield, B.V.Sc., M.R.C.V.S., in the issue of August/September, 1940, on page 333 it is erroneously stated that the specimen was supplied by Mr. E. Greenway, M.R.C.V.S., whereas it came from Mr. J. Adamson, M.R.C.V.S., of Bolton.

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